DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date December 13, 2001

From Biologist/Preclinical Reviewer

Subject Preclinical Issues Summary for P000058, InFUSE™ Bone Graft/LT-Cage Lumbar Tapered Fusion Device

To Advisory Panel members

The sponsor's device contains a purified recombinant cytokine that induces the formation of bone. This cytokine, recombinant human bone morphogenetic protein 2 (rhBMP-2) is a member of the TGF-β superfamily of growth factors and contains cysteine residues in amino acid sequence position characteristic of this family of proteins. rhBMP-2 is a glycosylated, disulfide-bonded, dimeric protein with two major subunit species of [redacted] and [redacted] amino acids, with the two chains differing in their N-termini due to differential processing. rhBMP-2 is synthesized as a large precursor of [redacted] amino acids which includes a hydrophobic secretory leader sequence ([redacted] amino acids) and a substantial propeptide region ([redacted] amino acids). After dimerization, the propeptide is cleaved from the precursor protein during cellular processing to yield a mature, covalent, dimeric species, consisting of the carboxyterminal portion of the promolecule. rhBMP-2 is expressed and secreted in a Chinese Hamster Ovary (CHO) cell culture process and subsequently highly purified using a 3-step chromatography process. Members of the TGF-related superfamily are present in a wide variety of species including insects, amphibia, and birds. The proteins are involved in the control of physiologic processes associated with cellular proliferation and differentiation (reference 1).

The sponsor has conducted an extensive array of preclinical experiments to evaluate the biocompatibility, safety and effectiveness of the product. The experiments are described in the preclinical summary provided by the sponsor in the panel package. FDA has concerns regarding 2 issues that we believe have not been completely evaluated. The concerns remaining regard:

a. the potential for rhBMP-2 to stimulate the proliferation of undetected transformed cells in patients implanted with the device; and

b. the potential for antibodies elicited by rhBMP-2 to cross the placenta and disrupt normal embryogenesis, and the potential for fetal BMP-2 to stimulate the maternal immune system.

As panel members of this advisory committee, you will be asked your opinion with respect to these issues. This memo will present the issues and will briefly summarize what the sponsor has done via preclinical evaluations to address the issues.

The potential for rhBMP-2 to stimulate the proliferation of undetected transformed cells in patients implanted with the device

Manufacturers of medical devices implanted for periods of time exceeding 30 days are advised to evaluate their product via cytotoxicity, sensitization, genotoxicity, implantation, chronic toxicity and carcinogenicity assays. The sponsor has identified in their preclinical summary (tables III-1 and III-4) those experiments conducted that were done to address these concerns. The experiments conducted directly addressed the cytotoxicity, sensitization, chronic toxicity, and implantation recommendations. The sponsor conducted one mutagenicity assay, the Ames Reverse Mutation Assay, and has referred to the International Conference on Harmonization (ICH) guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals which states that "the range and type of genotoxicity studies routinely conducted for pharmaceuticals are not applicable to biotechnology-derived pharmaceuticals and therefore are not needed".

The concern with respect to the effect of rhBMP-2 on tumor cell proliferation is more focused on the potential growth enhancing effect rhBMP-2 might have on BMP-receptor expressing cell types rather than the role of rhBMP-2 to initiate transformation (reference 2). The sponsor has conducted a number of short and long-term toxicity assays that demonstrate product safety. Most important of these assays, with respect to additional carcinogenicity information, was a 1-year Femoral Onlay Implant toxicology experiment done in Sprague-Dawley rats. No toxic effects or malignancies were observed at doses ranging from 0.04-1.6 mg/kg over a one year period.

Additionally, the sponsor referenced *in vitro* evaluations of the potential for rhBMP-2 to stimulate the growth of tumor cell lines and primary tumor isolates. The sponsor assessed the potential effects of rhBMP-2 to stimulate tumor cell line proliferation at 10, 100, and 1000 ng/mL. Human osteosarcoma cell lines (SaOS-2, U-2, OS, TE-85, and MG-63) were unaffected by treatment. Human prostate carcinomas (DU-145 and PC-3), breast carcinomas (ZR-75-1 and HTD-30), tongue carcinoma (SCC-9) and lung carcinoma (HTB-58) were all growth inhibited by rhBMP-2. The sponsor referred to a study published by Soda et al. in which primary tumor isolates were evaluated for their responsiveness to rhBMP-2. The effect of rhBMP-2 at 10, 100, and 1000 ng/mL on cell growth of the primary tumor isolates was assessed. In the study conducted by the sponsor no information regarding BMP-2 receptor expression of these cells is known. It is unclear how the effects on cellular proliferation are correlated with receptor expression. In the study by Soda et al. of the 113 specimens tested only 65 (57.5%) were evaluable (reference 3). Additionally, BMP receptor status also was not determined.

In summary of the concern of rhBMP-2's potential effect on transformed cells, we do not have information regarding its potential to stimulate transformed cells expressing BMP-2 receptors in an *in vitro* or in an *in vivo* setting. FDA communicated this concern to the sponsor (Sofamor Danek) in the following deficiency:

Following review of all information submitted in response to this issue, and considering the general health status of the proposed patient population, as well as the permanent nature of the treatment regimen, it is felt that additional studies are still needed to better assess the potential for rhBMP-2 to enhance the growth of pre-existing tumors. Prior to addressing this issue, we suggest that you contact us to discuss potential experimental methods. The following is one potential experimental approach:

a. The performance of *in vitro* assays using human primary cell cultures and a variety of human tumor cell lines to assess the expression of the receptor for BMP-2. Note that specimens representing prostate tumors and pancreatic tumors, as well as various types of osteosarcomas and tumors derived from bone metastases, should be studied.

b. The performance of *in vitro* assays using primary cell cultures and human tumor cell lines selected based on the *in vitro* receptor expression data, in order to evaluate various concentrations of BMP-2 upon tumor growth.

c. The performance of *in vivo* studies in mouse xenograft tumor models, using human tumor cell lines selected based on the *in vitro* studies. Such studies would investigate whether or not there is any enhancement of tumor growth by BMP-2, and if so, would obtain additional information about the dose-response for the findings seen.

The sponsor has submitted detailed protocols to FDA, and in general scope, the experimental designs are reasonable and address the issues of the deficiency.

FDA believes that the sponsor has demonstrated that the device is safe for use in preclinical and clinical studies. FDA believes that additional information can be obtained via post-approval studies/commitments by the sponsor investigating the potential for rhBMP-2 to stimulate transformed cell growth. The sponsor has agreed to these commitments. Until information has been obtained and it has been shown to more definitively demonstrate a lack of risk, FDA believes that the product labeling should specify the appropriate patient population for use of the product.

Please be prepared to discuss the degree to which the potential for rhBMP-2 to cause the promotion of growth of transformed cells in patients implanted with the sponsor's product is a concern, whether the requested studies should adequately address the concern, and what statements with regard to this concern should be provided on the product label.

The potential for antibodies elicited to rhBMP-2 to cross the placenta and disrupt normal embryogenesis.

BMPs are involved in multiple developmental processes during embryogenesis, and the proteins appear to be expressed in a developmentally regulated manner. BMP-2 through BMP-7 messenger ribonucleic acids (mRNAs) can be detected in a variety of locations other than the developing skeletal system, where mesenchymal-epithelial inductive interactions occur. BMPs have been shown to be specific in the specification of the ventral mesoderm and involved in the development of nearly all organs and tissues, including the nervous system, somites, lung, kidney, skin, and gonads, as well as in critical steps in the establishment of the basic embyonic body plan. Mice in which the gene for BMP-2 has been deleted, or knocked-out, are nonviable and have defects in amnion/chorion and cardiac development (references 4 [not included], 5, and 6).

The sponsor has conducted teratology experiments in rats and rabbits to evaluate the potential for their product to have a deleterious effect on developing embryos. No toxic findings were observed in the fetuses, dams, nor in parameters of reproduction. Although this information indicates that the cytokine did not cause obvious abnormalities in the species evaluated, the information does not address whether the absence of the cytokine during embryogenesis, as might occur due to a patient's immune response to the protein, could cause toxic effects in developing human embryos. FDA is concerned that antibodies elicited by rhBMP-2 could cross the placental barrier and effectively "knock-out" the embryos' own BMP-2 during a critical phase of development. There is a low incidence of patients who exhibit an immune response to rhBMP-2 among various clinical applications. An additional concern is whether expression of fetal BMP-2 could stimulate the immune system of women who previously had mounted an immune response to implanted rhBMP-2.

Please be prepared to discuss the degree to which there should be concern regarding transplacental transmission of maternal antibodies and their effect on the developing embryo, and the potential for a woman, previously implanted with the product, to respond immunologically to fetal expression of BMP-2. Other issues for discussion will include the design and possible use of a patient registry for women of childbearing potential who have received the product, what studies could be conducted by the sponsor to investigate these risks, and labeling recommendations.

References

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3. Soda, H., Raymond, E., Sharma, S., et al. (1998). Antiproliferative Effects of Recombinant Human Bone Morphogenetic Protein-2 on Human Tumor Colony-Forming Units. Anti-Cancer Drugs 9, 327-331.

4. Zhang, H., and Bradley, A. (1996). Mice Deficient for BMP2 Are Nonviable And Have Defects in Amnion/Chorion and Cardiac Development. Development 122, 2977-2986.

5. Ying, Y., and Zhao, G-Q. (2001). Cooperation of Endoderm-Derived BMP2 and Extraembryonic Ectoderm-Derived BMP4 in Primordial Germ Cell Generation in the Mouse. Developmental Biology 232, 484-492.

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